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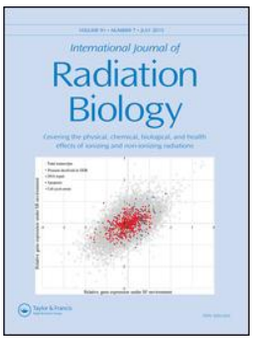

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Microbeam evolution: From single cell irradiation to preclinical studies

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Abstract

Purpose

This review follows the development of microbeam technology from the early days of single cell irradiations, to investigations of specific cellular mechanisms and to the development of new treatment modalities *in vivo*. A number of microbeam applications are discussed with a focus on preclinical modalities and translation towards clinical application.

Conclusions

The development of radiation microbeams has been a valuable tool for the exploration of fundamental radiobiological response mechanisms. The strength of micro-irradiation techniques lies in their ability to deliver precise doses of radiation to selected individual cells *in vitro* or even to target subcellular organelles. These abilities have led to the development of a range of microbeam facilities around the world allowing the delivery of precisely defined beams of charged particles, X-rays, or electrons.

In addition, microbeams have acted as mechanistic probes to dissect the underlying molecular events of the DNA damage response following highly localised dose deposition. Further advances in very precise beam delivery have also enabled the transition towards new and exciting therapeutic modalities developed at synchrotrons to deliver radiotherapy using plane parallel microbeams, in Microbeam Radiotherapy (MRT).

1. Introduction

Biological damage induced by ionising radiation occurs due to chemical changes caused by ionisation at the cellular level. The classical radiobiology paradigm is that nuclear DNA is the primary target for biological damage. The amount of biological damage induced by ionising radiation depends on variables including dose, the rate of absorption, the exposed area, and the variations in radical species, between specific tissues and cells, and in radiosensitivity between individuals (Joiner & van der Kogel 2009).

Ionising radiation has been successfully exploited in radiotherapy as a powerful cancer therapy, which has been significantly refined due to the accumulation of knowledge on its effects derived from new advances in epidemiology and radiobiology (Clement et al. 2012).

Continuous technological advances and new radiobiology challenges are behind the interest in the use of micro-irradiation techniques for radiobiological studies. Due to the very small beam size and highly precise targeting within the cell, microbeams have empowered researchers with unique investigative methods. In particular, the very precise dose delivery has played a fundamental role in the investigation of non-targeted effects where the radiation response is induced in cells which are not directly exposed to ionising radiation (Schettino et al. 2010). The technology has also recently contributed to the discovery of important novel time-sensitive interaction mechanisms of ionizing radiation with cells and tissues (Ghita et al. 2017; Walsh et al. 2017).

A number of technical features are vital to ensure that microbeams have the versatility and high specificity that is key for modern radiobiological experiments. These include the targeting accuracy, the particle counting efficiency, the dose rate and the rate at which cellular targets can be identified and irradiated. A wide range of facilities have been

developed worldwide delivering charged particles (including protons and helium ions), X-rays, and electrons for a number of specific *in vitro* and *in vivo* applications.

Depending on the beam origin, modern microbeams are divided into either cyclotron or accelerator based (for particle microbeams), compact X-ray source based (e.g. soft X-ray), and synchrotron based facilities.

For basic radiobiological experiments where individual cells can be targeted, the key components of a microbeam are shown in figure 1 and include beam transport and microbeam producing devices, radiation detection, beam control, and cell dish design. These can be implemented in a number of different ways depending on the specific application of the facility. Microbeams are typically also equipped with an imaging station allowing the users to identify targets and align them with the radiation probe. They can also be used for following up the dynamics of cellular processes such as DNA damage and repair in real time.

For beam size adjustment, different methods to reduce the beam size are employed depending on the target. Cell dish design and particle detection are strongly dependent on the beam species, carefully considering beam orientation, any possible beam scatter around the dish or the very thin penetration depth of the particular beam. These aspects have been previously discussed in an in depth review on technical aspects of microbeams (Schettino et al. 2010).

In an alternative approach, synchrotron based microbeams deliver X-rays emitted tangentially from relativistic electron bunches circulating in a storage ring. The irradiation modality consists of an array of microbeams (25-100 μm width), created by inserting a multi-slit collimator in the path of the high-flux Synchrotron X-Rays (Bräuer-Krisch et al. 2009; Bouchet et al. 2016; Smyth et al. 2016).

Microbeam facilities have been used in a variety of models to unravel some of the early events occurring immediately after the localized DNA damage within irradiated cells and in adjacent non irradiated populations both *in vitro* and *in vivo* (Richard et al. 2011), (Buonanno

et al. 2015) and (A Bertucci et al. 2009) . Recently, the technology has been used to elucidate bystander and abscopal effects *in vivo* (Fernandez-Palomo et al. 2015) using an array of microbeams and progressing towards the development of novel radiotherapy modalities: microbeam radiation therapy (MRT) (Bräuer-Krisch et al. 2015).

This review follows the development of microbeam technology from the early days of single cell irradiations to the development of new treatment modalities using microbeams focusing on preclinical developments and translation towards clinical applications

2. Particle microbeams

Microbeam approaches have been around since the early development of a UV microbeam by Tschachotin in 1912 (Wu & Hei 2017). The first particle microbeam experiment was performed by Zirkle and Bloom in 1953 (Zirkle, Raymond & William 1953) using a 2 MV Van de Graaf accelerator to generate energetic protons. Micro-collimators consisting of two metal plates, with a groove etched in one of them, were clamped together to achieve a beam size of 2.5 μm. This was used to study the process of cell division after proton exposure. From here, the development of modern microbeams has intensified in the early 1990s, with the early developments using the cyclotron facility at the Brookhaven National Laboratories used to simulate the biological effects of cosmic rays. These first observations of a dose-volume effect were the beginning of the later proposed MRT technique (W et al. 1959; Slatkin D N et al. 1992). Another pioneering study used the 2 MeV tandem accelerator-based microbeam at Pacific Northwest Laboratories (Braby 1991).

Single cell charged particle microbeams can be grouped into two main categories according to the approach used to reduce the radiation beam to sub-cellular dimensions: microbeams using a *collimation assembly* and facilities employing *electromagnetic focusing*.

Many of the early microbeams used collimation approaches, whereas electromagnetic focusing has recently become a more common approach reflecting both technological advances and the need for finer resolution to probe micron level interactions within cells (Schettino et al. 2010).

Collimators and apertures have been extensively used at pioneering facilities of modern radiobiological microbeams, including Pacific Northwest Laboratory, the Gray Cancer Institute and Columbia University. Using fused silica tubing with apertures as small as 1 μm diameters, 90% protons and 99% $^{3}He^{2+}$ were confined within a 2 μm spot (Melvyn Folkard et al. 1997). Also, laser drilled apertures of 5-6 μm were used to achieve 5 μm beams with 91% of non-scattered particles (Melvyn Folkard et al. 1997).

The *electromagnetic focusing* approach utilizes a variety of magnetic quadrupoles to obtain extremely narrow charged particle beams in vacuum. However, the focused beam has to be extracted in air, with a significant scattering induced by the vacuum window, air gap and traversal of the cell support membrane.

Electron microbeams rely on standard electron guns and electrostatic devices for beam generation. They produce and accelerate the energetic electron beams, which are subsequently reduced to micrometre size by the use of apertures or electromagnetic focusing (Sowa et al. 2005). As electrons undergo more scattering when interacting with biological samples compared to heavier charged particles, it is challenging for electron microbeams to achieve targeting resolutions at the micron or submicron level despite the size of the focused beam in vacuum. This poses challenges not only related to the resolution of electron microbeams but also to the calculation of the energy deposition and subsequent spatial dose distribution at the cellular level. These effects are not only significant for electron microbeams, but also for biological dosimetry of low energy electrons in general (Sowa et al. 2005; Siragusa et al. 2017).

An overview of the facilities currently in operation, dedicated to biology or shared with analytical experiments, has recently been presented (Barberet & Seznec 2015) and an update is shown in table 1.

3. X-ray microbeams

Considering the irradiation geometry, X-ray microbeams employ either a *single beam* or *an array of microbeams*. For cellular irradiation, single X-ray microbeams are used to specifically target subcellular compartments of the cells and analyse specific mechanisms behind cellular damage repair. However, there is growing interest in the preclinical setting of irradiation of *in vivo* tumours using arrays of microbeams with a more complex geometry and dose delivery. In addition to the beam geometry, the X-ray energy used and doses delivered will also vary between these two different types of microbeams (Folkard et al. 2001a) (Bouchet et al. 2010). A current list of soft X-ray and synchrotron microbeam facilities is presented in table 2.

Single X-ray microbeams have been developed, starting in the 1990s (Schettino et al. 1997) to provide quantitative and mechanistic radiobiological information to complement charged particle studies. Damage caused by X-rays delivered to a single cell is qualitatively different to lesions produced by charged particles due to reduced clustering of the ionizations (D. T. Goodhead 1994). As scattering is not as important, X-ray microbeams are, in theory, capable of achieving radiation spots of an order of magnitude or more, smaller than those achieved with ion beams. Moreover, such this high spatial resolution is maintained as the X-ray beam penetrates through cells making it possible to irradiate deeper targets with micron precision, making its therapeutic use feasible.

Modern X-ray microbeams employ benchtop based electron bombardment X-ray sources for energies from 278 eV to 4.5 keV (Schettino et al. 2000). In addition to characteristic

radiation, the electron bombardment of the target will produce a continuum of bremsstrahlung with a maximum energy equivalent to the energy of the incident electrons. This radiation is undesirable because it will not be focused correctly, and can be significantly more penetrating than the characteristic X-rays. The bremsstrahlung component is removed by reflecting the radiation off a silica mirror mounted between the target and the focusing assembly (Schettino et al. 2000) For this type of soft X-ray microbeams very small probes can be achieved by the use of X-ray optics developed for high-resolution X-ray microscopic imaging.

The finest X-ray probes have been obtained using zone plates. These are circular diffraction gratings with radially increasing line densities, in a fashion that brings the diffracted X rays to an axial focus (Folkard et al. 2001a). As with other diffraction devices, several diffracted orders are produced, and the unwanted orders must be prevented from reaching the cells, because they will not be appropriately focused. To do this, an arrangement of masks is used that allows only the first-order diffracted X-rays to reach the target. An important challenge when employing low energy microbeams is the attenuation in air requiring a very delicate dish design (Schettino et al. 2000).

Alongside the self- contained design of these devices, the advantage of electron and X-ray microbeams lies in the ability to easily vary the beam energy and therefore the LET. This enables a range of investigations in the context of relative biological effectiveness (RBE) for different energies. In this respect, electron and X-ray microbeams complement the work done with charged particle facilities to investigate the LET dependence (Folkard et al. 2001b; Wu & Hei 2017).

Synchrotron microbeams use much higher energies in the range of 2.34-600 keV (Crosbie et al. 2015; Kaminaga et al. 2016). The beamlines use a bending magnet (at low energies) or a wiggler (at higher energies) to produce a virtually parallel beam of X-ray with minimal

vertical divergence. This is then spatially fractionated using collimators and arranged in an array of alternating parallel micro-planar beams and gaps. This segments the high flux X-ray beam from a synchrotron into a micro-planar lattice of narrow beams, typically 25-50 μm wide and with a centre-to-centre separations of 200 or 400 μm. These can then potentially be cross-fired providing a large array of options for novel treatment modalities (Bouchet et al. 2010; Crosbie et al. 2010). This unique spatial distribution allows the delivery of an array of peak and valley doses. The former is directly deposited in the target by the microbeam while the latter is deposited in the tissue between the beams by scattered photons (Blattmann et al. 2005). The dramatic dose difference between heavily (peaks) and lightly (valleys) irradiated tissue, promoted when a broad beam is converted into an array of microbeams, is a very important characteristic unique to synchrotron microbeams. The peak dose delivered is typically up to 300-800 Gy at skin entry, with valley doses of 12-20 Gy and mean dose rates in the range of thousands Gy s^{-1} (Crosbie et al. 2010; Bräuer-Krisch et al. 2015).

This setup has been used to irradiate cells, tissue and small to medium sized animals in an experimental technique known as MRT, and a schematic representation of a typical MRT dose distribution is shown in figure 2. This modality was shown to have a preferential killing effect on tumour cells, which has been demonstrated in glioma models (Fernandez-Palomo et al. 2015; Smyth et al. 2016). Hypotheses for the efficacy of MRT suggest it is due to the periodically alternating dose distribution, proposing mechanisms based on observations including the preferential damage to tumour microvasculature compared to normal brain microvasculature *in vivo* (Bouchet et al. 2010; Bouchet et al. 2016); in-field bystander effects related to cellular migration *in vitro* and *in vivo* (Crosbie et al. 2010; Bouchet et al. 2017) and the communication of stress factors *in vitro* between peak and valley regions (Smyth et al. 2016). Research has also revealed that MRT seems to modulate the immune system by

regulating the expression of growth factors, cytokines and lymphokines (Bouchet et al. 2013) and the recruitment of tumour-associated immune cells (Yang et al. 2014).

4. Microbeam dosimetry challenges

A key feature of modern microbeam facilities is the ability to establish *a priori* an accurate reproducible dose that will be delivered to each sample. By coupling this with a high efficiency detection system, doses can be precisely monitored and controlled by very fast beam shuttering or deflection system (Melvyn Folkard et al. 1997).

Particle detection characteristics can be used to separate microbeams into two categories based on whether the detection occurs before or after the particles reach the biological sample (Schettino et al. 2010). By placing the detector between the vacuum window and the sample holder, no further constraints are imposed on the sample holder or the cell environment. However, the inevitable detector-beam interaction reduces the quality and accuracy of the exposure. In order to minimise the energy loss in the detector, only thin, transmission type detectors are appropriate. These detectors are generally thin film plastic scintillators which generate flashes of light when traversed by particles. These flashes are collected by a photomultiplier, and processed into individual particle counts (Melvyn Folkard et al. 1997).

An alternative configuration consists of placing the detector behind the sample holder. Using this approach, no extra scattering is introduced by the detector and better targeting accuracy can be reached. While conventional solid state detectors can be used, this configuration requires that the delivered particles have enough energy to pass through the sample, setting a limit on the lowest usable energy. In many cases it is also necessary to remove the culture medium requiring additional procedures to keep cells viable during the irradiation process (M Folkard et al. 1997) and (Randers-Pehrson et al. 2001).

Due to the small radiation beam and the localized delivery of the radiation dose, conventional dosimetry approaches are not always relevant for microbeam exposures when used for single cell or sub-cellular targeting. The dosimetry for the microbeam facilities is usually reported in terms of the number of photons/ions delivered to a specific biological target. The delivered dose depends on the particle species, energy along with detector efficiency and geometric characteristics of the cell (Folkard et al. 2001b). The timescale throughout which the radiation is delivered is also an important parameter, particularly for new radiation sources able to deliver high fluxes of radiation.

However, the number of particles (or photons) delivered to the target of interest is only the first dosimetric measurement which has its main advantages in a direct and relatively straightforward comparison of the samples irradiation record. In order to relate the radiation responses measured with microbeams to conventional radiation exposures, it is important to estimate the energy deposited in individual cells or specific sub-cellular targets. Such calculations require the number of radiation events experienced by the cell together with information about the cell geometry and radiation energy. Difficulties in defining the volume (and therefore mass) of interest makes it hard to report such dosimetry assessment in terms of macroscopic dose measurements (i.e. Gy) and has led to definition of parameters such as 'specific dose' which characterises the dose deposited in specific cellular sub-components (Randers-Pehrson 2002).

The dosimetry for synchrotron based microbeams is more complex and requires aspects of spectrum verification (Crosbie et al. 2015) and absolute dose measurements at ultra-high dose rates (Fournier et al. 2016). Standard protocols for high resolution Gafchromic film measurements in combination with microscopy have been used with an accuracy of better than 5% for the peak dose and between 10% and 15% for the valley doses (Bartzsch et al. 2015; Bräuer-Krisch et al. 2015). For MRT, the dose to a large macroscopic volume is

usually reported together with information about the size of the microbeams, the gap between the microbeams and the ratio of peak-to-valley dose (i.e. dose in the centre of microbeam compared to dose in the gap between the microbeams). Assessment of the dose in such small volumes is challenging and requires dedicated protocols and tools. A variety of detectors have been investigated for dosimetry assessment in synchrotron microbeams with each presenting both limitations and advantages (Bräuer-Krisch et al. 2010), (Alagoz et al. 2016), (Gagliardi et al. 2015), (Okada et al. 2011).

5. Cellular and tissue effects

The main focus of research conducted into the effects of ionising radiation on cells has focused on the damage to the cell nucleus and the detrimental effects this has on the cell. Much of radiation biology and radiotherapy builds on the assumption that a high enough dose of energy deposited to the nucleus will ultimately lead to cell death. Within this dogma the cytoplasm, the cellular environment in which most cellular processes take place, has rarely been taken into account. With the development of microbeam facilities, the role of the cytoplasm in radiation-induced biological responses became increasingly important in studies of cytoplasm targeted reactions (Walsh et al. 2017), bystander cellular responses, (Tartier et al. 2007) and in interactions with gold nanoparticles (Ghita et al. 2017).

Subcellular Targeting

The strength of the micro-irradiation technique lies in its ability to deliver precise doses of radiation to selected individual cells *in vitro* or to preselected targets within cells. The development of microbeams has allowed further dissection of cellular and molecular events in various experiments for DNA damage and repair (Kashino et al. 2004; Tartier et al. 2007; Richard et al. 2011; Ghita et al. 2017; Walsh et al. 2017). These studies have made use of

advances in microscopy to quantify the radiation induced stress at different subcellular levels and have made a significant impact on the understanding of mechanistic radiation responses in cells. Also, single cell approaches have also been very useful in establishing the radiation risks related to the crossing of a single and a precise number of α particles (Miller et al. 1999) and (Hei et al. 1997).

Microbeam technology has led the way to further innovation investigating cellular targeting and responses in cells but other approaches have also played a role. For example, studies using Auger electron emitters targeted to different cellular compartments tested their potential to induce non-targeted effects. The induction of these effects was found to be equally potent whether the Auger emitter was located in the cell membrane, in the cytoplasm or in the nucleus of the donor cells (Paillas et al. 2016). Although most of these studies agree on DNA being the most radiosensitive target, other cellular compartments also seem to be involved in both effects, especially the cell membrane (Kassis 2004; Pouget et al. 2008; Kassis 2011; Paillas et al. 2016).

During the past 10 years, there has been a shift away from a totally DNA-centric approach to include models that invoke complex signalling pathways in cells and between cells within tissues. Several newly recognised responses have been classified as so-called non-targeted responses (Tartier et al. 2007) in which biological effects are not directly related to the amount of energy deposited in the DNA of the cells traversed by the radiation.

Intercellular Communication

A major shift in our thinking about radiation effects has taken place with the finding that non-irradiated cells can respond biologically when their neighbours are irradiated, referred to as bystander responses. The ability to select individual cells or regions of tissues for localized irradiation is key to determining the role of intra- and intercellular signalling, and in depth reviews have been focusing on this aspect for *in vitro* work (Prise et al. 2010; Prise et al

1998; G Schettino et al. 2010). The development of single cell microbeams facilitated the evaluation of *in vitro* oncongenic potential in the bystander cell populations (Sawant et al. 2001). A similar approach was used to analyse the biological response in non-targeted cell populations relative to microbeam irradiated cells (Ponnaiya et al. 2004).

A recent study investigated the cell death and cell-cycle arrest of microbeam-irradiated cells and adjacent non-irradiated bystander cells in a human HeLa-Fucci spheroid culture with time-lapse imaging (Kaminaga et al. 2016). To our knowledge, this was the first real-time imaging of the dynamics of microbeam-irradiated and non-irradiated bystander cells. This was further developed in a study showing radiation-induced pro-inflammatory responses, including signalling in the NF-κB-COX-2 pathway, in a human 3-D organotypic skin culture exposed to modified X-ray fields (Acheva et al 2017).

However, experiments with cell monolayers and single cell irradiation do not take into account the complex cellular responses at the tissue level. Progression to 3D models can reproduce many of the tissue characteristics *in vivo* are therefore ideal targets for studying non-targeted effects using microbeams (Durante & Friedl 2011).

Advanced Tissue Models

Extending the present two-dimensional (2D) cell culture results to more complex models has been an important area of development for microbeam research. In recent years, 3D culture methods, such as spheroid cultures (*i.e.*, small aggregates of cells growing free of foreign materials) (Fennema et al. 2013; Ishiguro et al. 2017) and organoid technologies (*i.e.*, stem cell-derived 3-D cultures) (Lancaster & Knoblich 2014), have been developed to preserve the biological characteristics of the original tissues or organs better than conventional 2D monolayer cultures. This progress could contribute to the elucidation of the molecular mechanisms of radiation-induced bystander responses at the tissue level and has potential for

the development of new diagnostic and therapeutic radiation techniques (Belyakov et al. 2001; Belyakov et al. 2006), (Buonanno et al. 2015; Peng et al. 2017).

More *in vivo*-like culture methods, such as *ex vivo* tissue and organ cultures, also have potential as useful tools for microbeam research. Organotypic tumour tissue slice methods optimized for *ex vivo* culture would be useful for assessing not only tumour-specific drug responses but also microbeam-induced bystander responses (Vaira et al. 2010; Naipal et al. 2016). Some *ex vivo* organ culture techniques (*e.g.*, human hair follicle (Langan et al. 2015), mouse testis (Sato et al. 2011)) are likely to be applicable to microbeam research.

Although traditionally hampered by the limited range of particles and photons used, animal models are now also being used in microbeam studies using a single microbeam. So far, these studies focused on very small animals, such as silkworms (Fukamoto et al. 2007) and nematodes (A Bertucci et al. 2009) but can provide important insights on long-range non-targeted effects, beyond the possibility of 3D tissue targets (Durante & Friedl 2011).

Single microbeam approaches for targeting individual cells offer the possibility to follow the cellular processes in real-time post irradiation. A future advance will be to translate these approaches into *in vivo* models, particularly to investigate low dose biological consequences. Ideally, these studies need to consider both spatial and temporal responses from cellular r to functional biological changes (Schettino et al. 2010).

Preclinical applications of MRT

Finally, moving beyond mechanistic studies, synchrotron MRT has shown high therapeutic potential in small animal models of malignant brain tumours with a preferential effect on intracerebral 9L gliosarcoma vascular networks (Bouchet et al. 2010; Crosbie et al. 2010). In this context a recent study (Fernandez-Palomo et al. 2015) interrogated γH_2AX as a biomarker for dose deposition in the brain after synchrotron microbeam irradiation. This

study shows a direct correlation between the irradiation dose and induced foci for entry doses up to 350 Gy. Furthermore, a correlation between the microbeam foci track width and dissection time was observed at the highest dose with no significant change in the width of the microbeam tracks seen at lower irradiation doses. This suggests that radiation induced bystander effects have an impact on the cells exposed to both the high-peak doses and the dose gradient of the transition zone (Fernandez-Palomo, Bräuer-Krisch, et al. 2015).

As a comparison, different DNA damage patterns after irradiation with 2 Gy using C soft X-ray of a 2D monolayer is shown in figure 3a and 3b. In an *in vivo* setup, DNA damage induced after synchrotron irradiation of mouse cerebellum with an entry-dose of 350 Gy is shown in figure 3c and 3d (Fernandez-Palomo et al. 2015).

6. Clinical translation for therapy developments

Microbeam radiation therapy (MRT), an innovative pre-clinical radiotherapy technique using spatially fractionated synchrotron X-rays, has been shown to spare radiosensitive tissues such as mammal brains (Serduc et al. 2008; Bouchet et al. 2016). In MRT the tumour is irradiated by arrays of micrometre wide planar beams of unconventionally high doses of up to a few hundred Gy that are separated by several hundred micrometre wide low dose regions (Bräuer-Krisch et al. 2005). The major benefit of MRT over conventional radiotherapy approaches is associated with the dose volume effect where the utilization of a micrometre-scale treatment beam width leads to a higher radiation tolerance of normal tissue compared to tumour tissue (Bouchet et al. 2010), (Schültke et al. 2017; Serduc et al. 2008). Pre-clinical studies have demonstrated this advantage in several animal models, such as weanling piglets, duck embryos, and suckling and adult rats (Slatkin et al. 1995; Laissue et al. 1999; Dilmanian et al. 2002; Dilmanian et al. 2003; Dilmanian et al. 2005; Serduc et al. 2009; Van Der Sanden et al. 2010; Laissue et al. 2013; Bouchet et al. 2014). The skin has also been shown to tolerate

doses of 835-1335 Gy very well in MRT, far above of those used in pre-clinical studies (350 Gy) (Zhong et al. 2003). Moreover, the acute effects on skin produced by high MRT doses were similar to the effects of low doses of broad beam (Priyadarshika et al. 2011). Thus, the organ tolerance, particularly of the normal brain, could allow re-irradiation of the tumour.

MRT in small animal models has achieved therapeutic ratios clearly exceeding those obtained by homogeneous dose distributions delivered using conformal preclinical radiotherapy in a range of malignancies (Grotzer et al. 2015). Currently, the production of clinical microbeams can only be facilitated at large synchrotron facilities like the European Synchrotron (ESRF) in Grenoble and the Imaging and Medical Beamline (IMBL) at the Australian Synchrotron, due to the high beam flux and quality requirements. However, the possibility to use a conventional X-ray tube or carbon nanotubes (S. Wang et al. 2011) to produce microbeams for preclinical studies has also been explored. This study used an X-ray tube with a small focal spot and a specially designed collimator were used to produce microbeams for preclinical research (Bartzsch et al. 2016).

The growing interest in bright monochromatic and tuneable X-ray sources for use in imaging and radiation therapy has led to the collaboration of seven research institutes and industry partners in the ThomX project, to develop a compact Compton Backscattering Source (CBS) based in Orsay – France (Variola et al. 2014). The project aims to provide a fully operational hard X-ray CBS upgradable to be operated with a relatively reduced cost (Alagoz et al. 2016). Another recent study proposes another novel technical solution of line focus X-ray tubes, with the aim of clinical translation of MRT (Bartzsch & Oelfke 2017). Long term, this might enable the development of clinical microbeams without the need of a synchrotron.

Various biological mechanisms have been suggested to explain MRT's effectiveness. Strong evidence indicating that the different repair efficiencies of blood vessels in malignant and

healthy tissue is a key factor in explaining the differential effect of microbeams (Bouchet et al. 2010).

Given the clinical potential of MRT, robust normal tissue toxicity data, especially pre-clinical depth-dose data, must be collected in order to successfully translate these therapies to human clinical trials. While previous work employed computational modelling (Merrem et al. 2017), a lack of robust reference data means that further experimental studies on the geometric properties of vascular networks are necessary to improve the predictions of the model. Previous reviews summarized the available normal tissue toxicity data from MRT animal studies and have considered how they relate to current normal tissue toxicity data and clinical dose constraints (Smyth et al. 2016). Furthermore, a novel treatment planning environment for synchrotron MRT has been developed based on the Eclipse™ treatment planning system (Poole et al. 2017). This is an essential step in MRT progression towards human clinical trials, as it is necessary that MRT not only meets current clinical standards but also has similarity with all stages of the radiotherapy process (Grotzer et al. 2015).

As part of the development of a clinical case for MRT, candidate populations for potential clinical trials have been discussed. Two key sites, in adults with glioblastoma multiforme and in paediatric patients with diffuse intrinsic pontine glioma, have been identified (Grotzer et al. 2015).

The phase 1 clinical trials in Synchrotron Stereotactic Radiotherapy (SSRT) have allowed the community to move forward with synchrotron based therapies requiring the implementation of a small hospital like environment at the biomedical beamline ID17 at the ESRF. With the SSRT clinical trial it will be possible to refine a protocol for dose enhancement using high Z elements in combination with low energy synchrotron X-rays. This can also be further exploited to improve the tumour control probability (TCP) in MRT (Bräuer-Krisch et al. 2015; Grotzer et al. 2015).

7. Summary and future directions

Microbeams have played a pivotal role in radiobiology. In its early days, the technology aimed to explore basic radiobiological effects after cellular irradiation. With the ongoing evolution of the field, microbeams have been refined to assess a range of responses after irradiation with X-ray or charged particles. The main advantage of the technology, the very controlled irradiation of micron-sized areas of tissue, has initiated novel research avenues beyond the investigation of the underlying mechanisms of radiotherapy. Microbeam technology has also served as the underpinning for MRT - a technique with the potential to impact the clinical landscape. Since the first reports using very small targeted radiation beams, the theoretical possibility of radiosurgery by irradiating parallel arrays of micro slices and cross-fired through tumours from several ports has attracted the attention of several groups internationally.

While the MRT concept has also been extended to proton heavy ion therapies, the development towards its clinical implementation is still a focus of radiation therapy programmes aiming towards its integration in the hospital environment (Bravin et al. 2015). With the increased number of potential applications of these technologies, novel technical and medical physics developments are key to further implement these methods into a clinical environment.

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Disclosure Statement

The authors report no conflict of interest.

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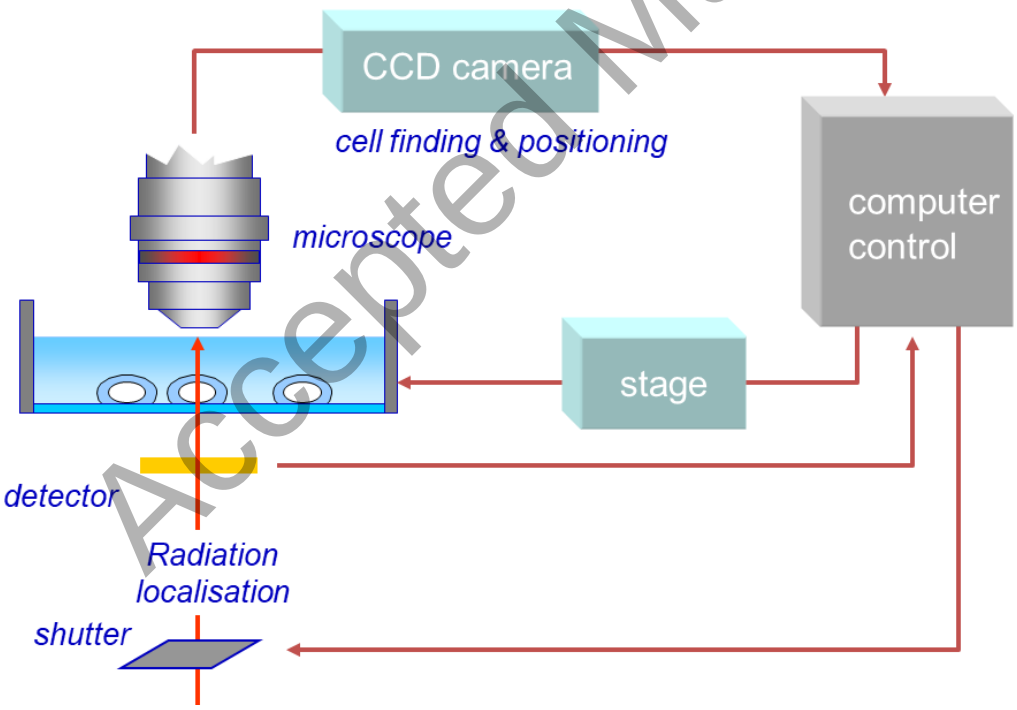

Figure 1 *Key accessories for a single cell irradiation a microbeam*

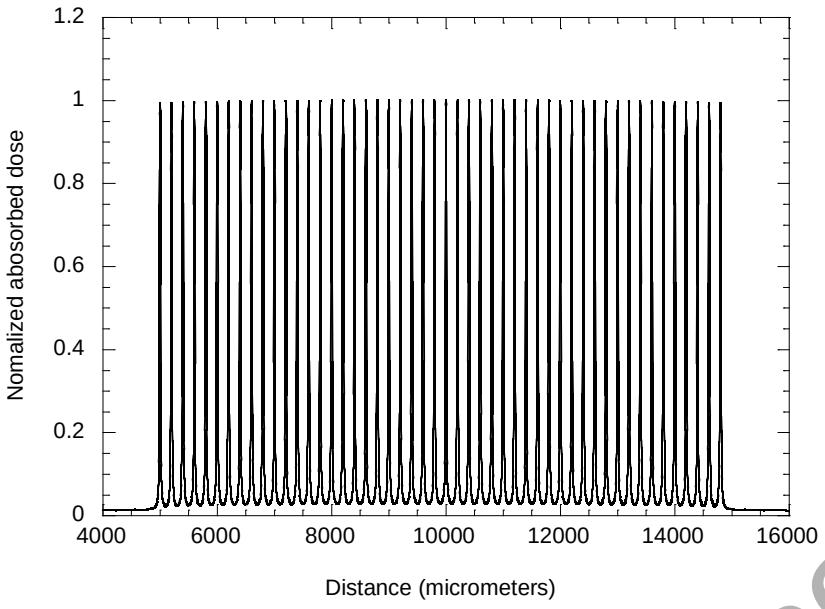

Figure 2 *Calculated lateral normalized dose profile for classical microbeam irradiation showing very steep dose gradients between peak doses and low doses delivered in the dose-valley regions.*

Table 1 *Updated list of particle microbeam facilities after* (Barberet & Seznec 2015)

Facility	Particle	Energy Range	Reference
RARAF Columbia University	Protons, α	1-5 MeV	Randers-Pehrson *et al* 1996 (Buonanno et al. 2015)
SPICE NIRS Chiba	Protons	3.4 MeV	Konishi *et al* 2013 (Konishi et al. 2013)
Ion Beam Centre Surrey	Protons, α, up to Ca	1-12 MeV	Merchant *et al* 2012 (Merchant et al. 2012)
IMP Fudan	Protons, α	6 MeV	Wang *et al* 2011 (X.F. Wang et al. 2011)
CENBG Bordeaux	Protons, α	1-3.5 MeV	Bourret *et al* 2014 (Bourret et al. 2014)
PTB Braunschweig	Protons, α	2-20 MeV	Mosconi *et al* 2011 (Mosconi et al. 2011) Patrono et al 2015 ((Patrono et al. 2015)
RIKEN Wako	Protons, α	3-4 MeV	Iwai *et al* 2008 (Iwai et al. 2008)
SNAKE Munich	Protons, α, Li, O, Si, Cl, I	4-28 MeV 1-10.5 MeV u^{-1}	Hauptner *et al* 2004 (Dollinger et al. 2005) Drexler *et al* 2015 (Drexler & Ruiz-Gómez 2015)
GSI Darmstadt	Protons, α, C to U	1.4-11.4 MeV u^{-1}	Heiss *et al* 2006 (Heiss et al. 2006)
Jaeri Takasaki	A, C, Ne, Ar	12.5-17.5 MeV u^{-1}	Funayama *et al* 2005 (Funayama et al. 2005)
Leipzig	H, He	2.25 MeV	Butz *el al* 2000 (Butz et al. 2000)

Electron microbeam Pacific Northwest National Laboratory	Electrons	Variable energy	Sowa *et al* 2005 (Sowa et al. 2005)

Table 2 *An updated list for X-ray microbeam facilities*

Facility	**Energy Range**	**Reference**
Columbia University	Ti soft X-ray 4.5 keV	Harken *et al* 2011 (Harken et al. 2011)
Brookhaven National Laboratory	2.584 GeV	Dilmanian *et al* 2001 (Dilmanian et al. 2003)
Queen's University Belfast	K-shell C soft X-ray 287 eV	Folkard *et al* 1997 (Folkard et al. 2001b) M. Ghita *et al* 2017 (Ghita et al. 2017)
European Synchrotron (ESRF), Grenoble	27-600 keV	Alagoz *et al* 2016 (Alagoz et al. 2016)
Australian Synchrotron Imaging and Medical Beamline (IMBL)	125 keV	Crosbie *et al* 2010 (Crosbie et al. 2010) Gagliardi *et al 2015* (Gagliardi et al. 2015)
Institute of Cancer Research	225 kVp	Bartzsch *et al* 2016 (Bartzsch et al. 2016)
KEK IMSS Photon Factory	2.34 keV	Kaminaga *et al* 2016 (Kaminaga et al. 2016)

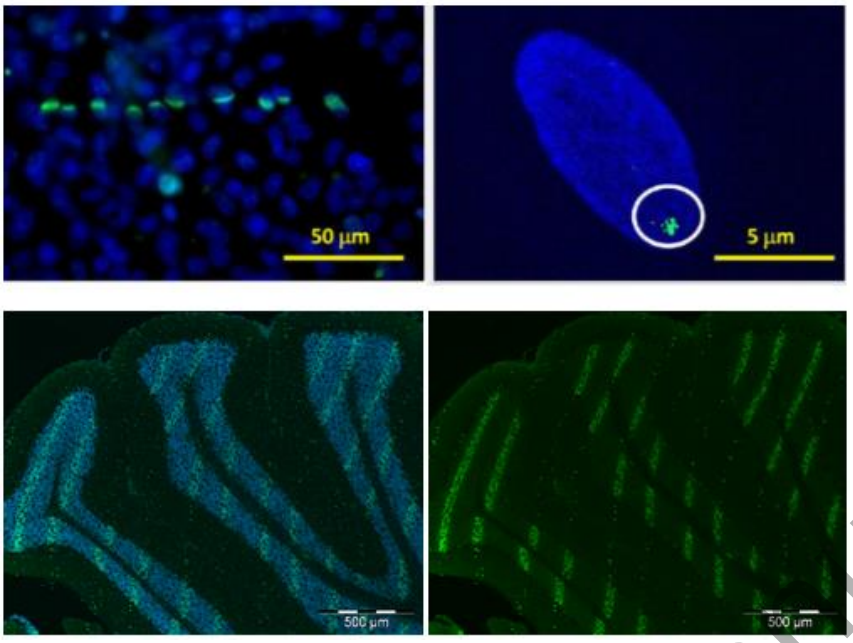

Figure 3 *Different magnitude of biological effects measured using γH$_2$AX used as a DNA damage marker after microbeam irradiation using C K-shell Soft X-ray used a) in scanning mode and b) with a dose of 2 Gy measured and delivered to cell nucleus in the outlined region; synchrotron X-ray used to target the cerebellum in c) and d), with an entrance dose of 350 Gy*